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Mismatch Repair*

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Highly conserved MutS homologs (MSH) and MutL homologs (MLH/PMS) are the fundamental components of mismatch repair (MMR). After decades of debate, it appears clear that the MSH proteins initiate MMR by recognizing a mismatch and forming multiple extremely stable ATP-bound sliding clamps that diffuse without hydrolysis along the adjacent DNA. The function(s) of MLH/PMS proteins is less clear, although they too bind ATP and are targeted to MMR by MSH sliding clamps. Structural analysis combined with recent real-time single molecule and cellular imaging technologies are providing new and detailed insight into the thermal-driven motions that animate the complete MMR mechanism.

Mismatched nucleotides in DNA can result from polymerase misincorporation errors, recombination between heteroallelic parental DNAs, and chemical or physical damage to nucleotides. MMR[2] was conceived simultaneously in 1964 by Evelyn Witkin to explain brominated nucleotide processing in bacteria and by Robin Holliday to explain gene conversion in yeast (1, 2). A DNA excision-resynthesis reaction was envisioned that would degrade one strand and use the complementary strand as a repair template to eliminate the mismatch (Fig. 1*A*). A genetic basis for MMR was established when the *hexA* mutation of *Pseudomonas* was found to be defective in gene conversion (3). The HexA gene turned out to be a homolog of the "Siegel Mutator" (MutS) of *Escherichia coli* (4), which with the *Salmonella LT7* Mutator (MutL (5)), the Hill Mutator (MutH (6)), and MutU (UvrD; Fig. 1*A*) (7) added to a growing list of genes, with historical roots in the 1954 description of the "Treffers Mutator" (MutT) (8). Mutation of these *Mut* genes substantially elevated spontaneous mutation rates (hence the designation as a Mutator). Today, most of the *Mut* genes are known to play a role in genome maintenance (9).

MutS, *MutL*, *MutH*, and *UvrD* were connected to MMR of polymerase misincorporation errors in 1980 (10). Faithful excision of the error-containing strand was found to target the unmethylated strand of a newly replicated hemimethylated (hm) DNA adenine methylation (Dam) GATC site (Fig. 1*A*) (11). Unfortunately, Dam-instructed MMR only operates in a subset of γ-proteobacteria such as *E. coli*. The mechanism of strand discrimination in eubacteria, archaea, and eukaryotes remains uncertain. Not surprisingly, the core MutS homologs (MSH) and MutL homologs (MLH/PMS) are highly conserved throughout the taxonomic domains, although some cellular functions have diverged with evolution (Fig. 1*A*; Table 1).

In 1993, the human HsMSH2 gene was linked to the common cancer predisposition Lynch syndrome or hereditary non-polyposis colorectal cancer (LS/HNPCC (12)). That observation was rapidly verified with the association of other MSH and MLH/PMS genes to LS/HNPCC and sporadic cancers (for review, see Ref. 13). These discoveries solidified a role for MMR in human tumorigenesis and provided support for the hypothesis that Mutators might be driving the large numbers of mutations found in cancer (14, 15). It also started a campaign to connect any gene remotely associated with DNA metabolism to genome instability. Interestingly, The Cancer Genome Atlas (TCGA) data clearly shows that most mutations found in human tumors are single base substitutions (16). The implication of these results is that an identifying feature of genuine drivers of genomic instability should be the production of single base substitutions or at least a demonstration of altered *mutation rates*.

In addition to MMR, the core MSH and MLH/PMS machinery has been linked to DNA damage signaling (17, 18) as well as the suppression of recombination between partially homologous parental DNAs (termed: hom*eo*logous recombination (19)). Although studies have shown that MMR is coupled to S-phase, both damage signaling and homeologous recombination are not tied to DNA replication (20). While this review will not discuss the mechanisms of DNA damage signaling and homeologous recombination (see Ref. 19), the possibility that MSH and MLH/PMS proteins have fundamentally different biophysical functions in these processes seems unlikely.

Mismatch Repair *in Vitro*

The random nature of polymerase misincorporation errors has made mechanistic studies of MMR *in vivo* difficult and dependent on biochemical analysis. Reconstitution of the *E. coli* (Ec) MMR reaction began in 1983 and utilized a DNA substrate containing two overlapping restriction enzyme sites with a central mismatch (Fig. 1*B*) (21). Strand specificity of DNA excision-resynthesis was easily determined based on which of the two initially restriction-resistant sites was used as a template during MMR. The repair reaction was dependent on a nearby hmGATC site (21) and was found to be bidirectional in that excision could be initiated either 3′ or 5′ of the mismatch depending on the location of the hmGATC site (22). In 1989, a complete system was reconstituted with purified components that, in addition to EcMutS, EcMutL, EcMutH, and EcUvrD, included single-stranded binding protein, the polymerase III

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[2] The abbreviations used are: MMR, mismatch repair; MSH, MutS homolog; MLH/PMS, MutL homolog; Mut, Mutator; hm, hemimethylated; Dam, DNA adenine methylation; ADP-BeF_2, adenosine 5′-beryllium fluoride; ATPγS, adenosine 5′-*O*-(thiotriphosphate); AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; EXOI, exonuclease I; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; Ec, *Escherichia coli*; Sc, *Saccharomyces cerevisiae*; Hs, *Homo sapiens*; SM, single molecule.

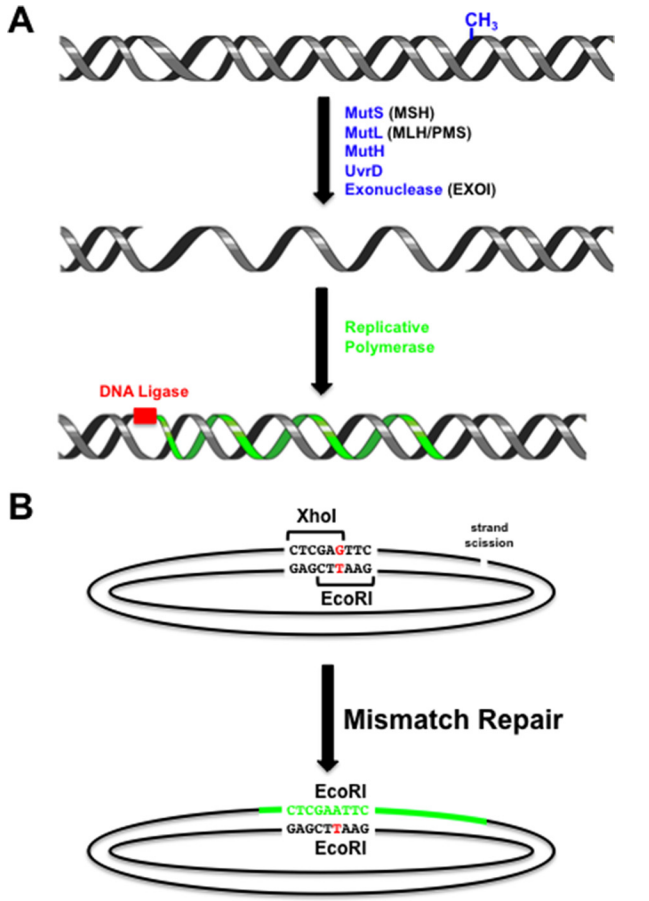

FIGURE 1. **The mismatch repair reaction.** *A*, illustration of the MMR excision-resynthesis process. The γ-proteobacteria components that direct strand-specific excision are shown in *blue*; bacterial (outside γ-proteobacteria), archaeal, and eukaryotic components are shown in *black*. The resynthesis on the exonuclease gap is performed by the replicative polymerase, and the remaining strand scission was sealed by DNA ligase. *B*, diagram of a simple MMR DNA substrate containing overlapping restriction sites containing a mismatch that result in resistance to endonuclease restriction. Strand scission-directed excision-resynthesis results in replacement of one strand and a gain of restriction sensitivity (EcoRI) that is diagnostic for which strand was used as a template.

holoenzyme complex, and DNA ligase (23). The 3′→5′ EcExoI exonuclease was used in the original reconstitution because the hmGATC was 3′ of the mismatch. Later genetic analysis showed that EcExoI was one of four redundant exonucleases associated with *E. coli* MMR (24). The other exonucleases included the 5′→3′ EcRecJ, the bidirectional EcExoVII, and the 3′→5′ ExoX, which together accounted for bidirectional MMR excision (24). These studies also convincingly demonstrated that EcMutS, EcMutL, EcMutH, EcUvrD, and an exonuclease exclusively instigated the MMR excision reaction. Single-stranded binding was required to protect the nascent ssDNA tract, which started at the hmGATC site and continued to just past the mismatch (25). Gap resynthesis and sealing appeared completely independent of the MMR excision process and did not require any special components outside the polymerase III holoenzyme complex and DNA ligase.

Reconstitution of eukaryotic MMR followed soon after the recognition that eukaryotic MMR required a preexisting strand scission (26). Interestingly, the eukaryotic reaction appeared nearly identical to *E. coli* in that it was bidirectional, and the excision tract began at the strand scission and continued to just past the mismatch (27). However, the eukaryotic MMR excision components for the 3′- and 5′-reactions were not identical (28–31). Like *E. coli*, 3′-excision required an MSH (MSH2-MSH6), an MLH/PMS (MLH1-PMS2), an exonuclease (EXOI), and the single-stranded binding heterotrimer RPA. Unlike *E. coli*, both the eukaryotic replicative processivity clamp PCNA and its clamp loader replication factor C (RFC) were found to be essential for 3′-excision, independent of the replicative polymerase or DNA synthesis (32). In contrast, the 5′-excision reaction appeared much simpler, requiring only an MSH (MSH2-MSH6), EXOI, and RPA. Interestingly, there was no helicase requirement for either the 3′-eukaryotic or the 5′-eukaryotic MMR reaction. This distinction almost certainly reflects the significant differences in MMR exonucleases; eukaryotic EXOI will initiate excision at a dsDNA strand scission, whereas the *E. coli* exonucleases act only on ssDNA.

MMR Model Version 1.0

By the mid-1980s, it was clear that the distribution of Dam GATC sites in *E. coli* was relatively random, which meant that the distance between a mismatch and the excision initiation site could be several thousand base pairs. The obvious question was: How does the MMR system communicate mismatch recognition to a distant excision initiation site?

Initial biochemical analysis showed that EcMutS recognized mismatch nucleotides (33), whereas EcMutH recognized and introduced a strand scission at a hmGATC site (34). Purified EcMutL increased the footprint of EcMutS on mismatched DNA (35), interacted with the EcUvrD helicase (36), and activated the EcMutH hmGATC endonuclease (37, 38). A pre-existing strand scission completely eliminated the EcMutH requirement for MMR *in vitro* (23), effectively converting it into a reaction that would eventually be recognized as similar to eukaryotes.

These activities fit nicely into a relatively straightforward Hydrolysis-dependent Translocation Model (Version 1.0) that solved the "distant-initiation" question by proposing that the EcMutS and EcMutL proteins formed a stable complex at the mismatch, creating a motor that used the energy of ATP hydrolysis to pull the DNA from both sides of the mismatch into a loop (39). In time, the motoring would presumably encounter an hmGATC site and recruit EcMutH to introduce a strand scission. That incision event would be followed by conscription of EcUvrD to unwind the DNA and present it to an ssDNA exonuclease for strand excision back toward the mismatch.

Although consistent with biochemical paradigms of the era, several perplexing mechanical issues were immediately apparent. For example, how would control of the opposing EcMutS·EcMutL motor driving along the DNA away from the mismatch be managed with the EcUvrD DNA helicase motor driving unwinding and excision toward the mismatch? How would a single large multi-protein assembly remain stable for the many minutes required to motor successively from the mismatch to a strand scission and then recruit more components to excise hundreds to thousands of nucleotides back to the mismatch?

TABLE 1
Conservation of mismatch repair components
Bold text indicates original bacterial MMR genes. IDL, insertion-deletion loop-type mismatches; ICL. inter-strand crosslink repair; SSA, single-strand annealing recombination repair; HR, homologous recombination.

E. coli	*S. cerevisiae*	Human	Function	Role
MutS	ScMsh1 ScMsh2-ScMsh6	—	Recognition of mismatches	Mitochondrial MMR
	HsMSH2-HsMSH6	Recognition of mismatches and small IDLs; Sliding clamp	MMR; ICL repair; gene conversion; heteroduplex rejection	
	ScMsh2-ScMsh3	HsMSH2-HsMSH3	Recognition of large IDLs and branched structures; sliding clamp	MMR; 3′-flap processing; SSA and triplet-repeat intermediate stabilization
MutS2[a]	ScMsh4-ScMsh5	HsMSH4-HsMSH5	Recognition of Holliday junctions; Holliday junction intermediates; sliding clamp linking two DNAs	Meiosis I; chromosome pairing and segregation
MutL	ScMlh1-ScPms1	HsMLH1-HsPMS2	Downstream mediator; endonuclease	MMR; gene conversion
	ScMlh1-ScMlh2	HsMLH1-HsPMS1	?	?
	ScMlh1-ScMlh3	HsMLH1-HSMLH3	MMR (?); downstream mediator of MSH4/5 sliding clamps; endonuclease (?)	Meiosis; crossover mediator
MutH				
UvrD	ScSgs1	HsRECQ1HsBLM HsWRN	Structure-selective DNA helicase	MMR (*E. coli* only); unwinding DNA during recombination
RecJ[b]	ScExo1	HsEXO1	5′→3′ exonuclease	MMR; HR
ExoVII			Bidirectional exonuclease	MMR (*E. coli*)
ExoI, ExoX			3′→5′ exonuclease	MMR (*E. coli*)

[a] Not present in *E. coli* but present in several eubacteria (see Molloy (101)).
[b] Directional but not functional or structural conservation with EXOI.

Biochemical analysis determined that the prokaryotic and archaeal MSH and MLH/PMS proteins functioned as homodimers, whereas the eukaryotic homologs functioned as heterodimers expressed from divergent genes that also evolved extended mismatch, lesion, and structure recognition properties (Table 1) (40–43). Evidence that EcMutS formed a tetramer was used to support complex formation and bidirectional movement in the Hydrolysis-dependent Translocation Model (94). However, mutations that specifically block tetramer formation have no effect on MMR *in vivo* (44), and there is no evidence that any other MSH form tetramers.

Conformations and Structures of MMR Proteins

The MSH proteins are related to the AAA^+ family of ATPases and contain a highly conserved Walker A/B nucleotide-binding motif (45, 46). In 1997, the human HsMSH2-HsMSH6 ATPase was shown to be controlled by mismatch-provoked ADP→ATP exchange (47). This property appeared similar to GDP→GTP exchange by G-protein molecular switches (48). That observation was followed by studies that showed MSH ATP binding resulted in the formation of a hydrolysis-independent sliding clamp that freely diffused along the DNA (49).

Asymmetric ATP binding, hydrolysis, and product release between MSH subunits were observed and ultimately determined to restrain unregulated ADP→ATP exchange (50–53). Misunderstanding the functions of asymmetric MSH ADP/ATP processing led to the persistence of the Hydrolysis-dependent Translocation Model because one could imagine alternating ATP binding and hydrolysis by subunits as a mechanism for inchworm-like movement along a DNA strand (MMR Model Version 1.1) (94). However, mismatch-, lesion-, or structure-provoked ADP→ATP exchange that results in the formation of *freely diffusible* ATP-bound sliding clamps is a central feature of all MSH proteins examined to date (38, 42, 43, 47, 49), which appears largely inconsistent with both MMR Model Version 1.0 and MMR Model Version 1.1.

Remarkably similar structures of MSH proteins bound to mismatched DNA have emerged (54–57). In all cases, there is a clamp-like configuration with a highly conserved Phe residue interrogating the DNA 3′ of the mismatch that obligatorily induces a 45–60° bend in the backbone. Only nucleotide-free or ADP-bound structures have been crystallized. Infusion of ATP or ATPγS destroyed the crystals (56), consistent with additional unresolved protein conformations. Several MSH structures infused with ADP-BeF_2 or AMP-PNP appear to trigger modestly altered structures (58). However, biochemical studies have shown that MSH proteins either do not bind AMP-PNP or remain bound to the mismatch, unable to form of a sliding clamp (47, 53), suggesting that these structures do not represent a *bona fide* ATP-bound MSH.

The shared function(s) of MLH/PMS proteins in MMR has been less transparent. MLH/PMS contain a gyrase, Hsp90, histidine kinase, Mutl (GHKL) superfamily ATP-binding motif (59) and an extremely weak ATPase activity that is required for MMR (60, 61). Atomic force microscopy has suggested that the ScMlh1-ScPms1 heterodimer undergoes ATP-dependent conformational contractions between the C-terminal dimer-heterodimer interaction domain and the N-terminal ATP-binding

and ATP-dependent dimerization domain (62). The function, if any, of these conformational transitions is unknown. EcMutL and ScMlh1-ScPms1 have been shown to bind ssDNA in very low ionic strength conditions (63, 64). However, this activity becomes nearly undetectable at physiological ionic strength (64). ATP binding by EcMutL enhances the EcMutH endonuclease activity (38). However, MLH/PMS ATP binding is not required to form a stable complex with MSH sliding clamps (38, 65).

MLH/PMS proteins outside γ-proteobacteria were found to contain an intrinsic ATP-stimulated endonuclease activity (66). It has been suggested that the MLH/PMS endonuclease might substitute for the MutH endonuclease. This parallel seems rather unlikely because the MLH/PMS endonuclease appears to introduce multiple strand scissions during MMR (66). The MLH/PMS endonuclease is most efficient in the presence of manganese divalent cation and may also be modestly stimulated by zinc (66). The divalent cation requirement of the MLH/PMS endonuclease *in vitro* remains puzzling because the abundance of manganese *in vivo* would appear insufficient to support significant activity. However, as might be predicted, the *Thermus thermophilus* TtMutL endonuclease is only activated upon its association with ATP-bound TtMutS sliding clamps (67).

PCNA loaded onto DNA by RFC significantly stimulates yeast and human MLH/PMS endonuclease activity (30, 66). Moreover, the orientation of PCNA appears to influence the directionality of the MLH/PMS endonuclease (68). These observations are consistent with specific interaction surfaces between the MSH·MLH/PMS complex and PCNA. How these surfaces support unambiguous 3′- and 5′-excision following the apparently random loading of MSH sliding clamps and subsequent specific complex formation with MLH/PMS remains an important question.

Initiating MMR Outside γ-Proteobacteria

Where does the strand scission arise that targets MMR outside of γ-proteobacteria? Recently, it was suggested that misincorporated ribonucleotides during replication may be the source of strand-specific breaks (69, 70). The idea is that the RNase H2 (RTH2) removes misincorporated ribonucleotides during S-phase, leaving a strand scission on the newly replicated DNA strand that might then faithfully direct MMR excision. Unfortunately, this hypothesis does not account for the observation that ribonucleotides are incorporated on average every 6–8 kb during replication (71), which appears significantly longer than MMR excision tracts, and the Mutator phenotype of *rnh2* mutations is at least 100-fold less than authentic MMR gene mutations (*e.g. mutH* mutations have an approximately equivalent Mutator phenotype to *mutS* and *mutL* mutations (72)).

A competing hypothesis proposes that remnant leading and lagging strand scissions that are left in the DNA following replication are used to direct replication-coupled MMR. This idea is consistent with the historical observation of persistent strand scissions associated with Okazaki fragments on the lagging strand, as well as the requirement of replication processivity clamp PCNA to direct MLH/PMS endonuclease activity for 3′-excision.

Real-time Single Molecule Imaging

The MMR protein structures appearing in the literature at the turn of the millennium led to the Static Transactivation MMR Model (Version 2.0). It was based on marrying the crystal surfaces of individual MMR proteins and proposing the formation of a static MSH·MLH/PMS complex on the mismatch. The distant-initiation problem was solved by envisioning that the complex could capture a looping strand scission via a three-dimensional (3D) collision (73). However, placing a stable biotin-streptavidin roadblock between the mismatch and strand scission site completely inhibited MMR, effectively eliminating this model and clearly implicating some type of DNA translocation process (74, 75). These studies underscored a major problem with model building based on static crystal structures that continues until now (55). It has also ushered in the era of real-time single molecule (SM) imaging (76), which has highlighted the importance of the vigorous thermal motions that ultimately animate biology (77–79).

At least three dynamic and functionally distinct forms of MSH have been visualized on DNA containing a mismatch by real-time SM imaging (Fig. 2*A*). Tracking *Thermus aquaticus* TaMutS showed that it formed an incipient clamp while searching for a mismatch. This TaMutS-searching clamp exhibited facilitated one-dimensional (1D) rotational diffusion while in continuous contact with the helical backbone (78). In effect, a searching TaMutS moved along the DNA much like a nut rotating on a screw. At physiological ionic strength, this search lasts for ~1 s and is calculated to examine ~1000 bp of naked DNA. A similar mismatch search mechanism was theorized for the ScMsh2-ScMsh6 heterodimer (76) and is likely conserved in all MSH proteins.

When an MSH encounters a mismatch, it pauses for ~3 s (Fig. 2*A*) (79). One imagines that this pause is required to form the static clamp exhibited in structural studies, which then provokes ADP→ATP exchange (38). Nearest neighbor analysis coupled with NMR has suggested that enhanced MSH mismatch recognition is tied to DNA flexibility surrounding the mismatch (80). One could easily envision dynamic thermal bending at the mismatch, compared to a normally smooth DNA backbone, as the distinction that elicits the pause in MSH diffusion. The detection of DNA contour alterations and not the mismatch itself would explain the wide range of mismatch/lesion recognition properties exhibited by MSH proteins (80). Although altered nucleotide stacking has been suggested to account for MSH recognition (81), the hierarchy of mismatched nucleotides that activates the MSH ATPase appears exactly opposite to that expected for such a conclusion (80). It is more likely that mismatch-induced changes in nucleotide rise, twist, tilt, and roll ultimately increase the degrees of freedom of glycosidic and phosphate bonds, enhancing DNA thermal flexibility (Ref. 80) and references therein).

Consistent with bulk studies (49), ATP binding resulted in the real-time observation of an MSH hydrolysis-independent sliding clamp (Fig. 2*A*) (78, 79). Also, as predicted, the release of one MSH sliding clamp from the mismatch allowed the loading

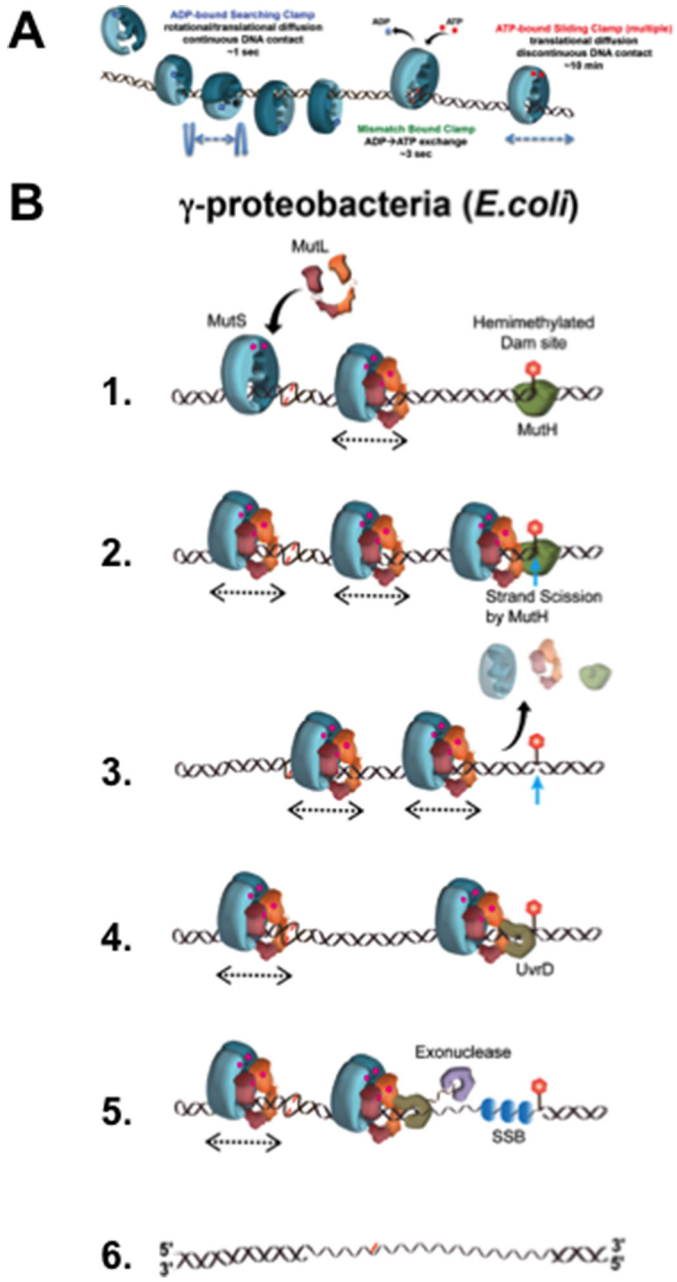

FIGURE 2. **The molecular switch model.** *A*, common MSH transitions during the mismatch search, recognition, and ATP-bound sliding clamp formation for all known organisms. From *left* to *right*: mismatch searching MSH, mismatch-bound MSH, and ATP-bound MSH sliding clamp. Diffusion characteristics and dwell times are detailed above/below each transition state. See text. *B*, downstream interactions of γ-proteobacteria such as *E. coli*. ***1***) The formation of multiple ATP-bound MutS sliding clamps (*A*) attracts MutL, which diffuses along the DNA as multiple MutS·MutL complexes. ***2***) The interaction of one MutS·MutL complex activates MutH, which introduces a strand scission on the unmethylated strand of a GATC Dam methylation site. ***3***) Following MutH incision, the MutS·MutL·MutH complex spontaneously dissociates. ***4***) A following MutS·MutL sliding clamp complex interacts with UvrD, which is attracted to the single-strand scission and stabilizes its DNA binding. ***5***) The MutS·MutL clamp complex enhances the processivity of the UvrD helicase, allowing strand unwinding and presentation of the single-stranded DNA to one of the four MMR exonucleases. *SSB*, single-stranded binding. ***6***) The process in ***B5*** is iterative until the mismatch is released, eliminating the loading of additional MutS sliding clamps. See text.

of multiple MSH sliding clamps that diffuse independently along the DNA (49, 78). The first SM imaging surprise was that ATP-bound MSH sliding clamps were incredibly stable on the DNA, with a lifetime of ~10 min (79). The second surprise was that the thermal diffusion mechanics were quite different when compared with an MSH searching for a mismatch (78). Instead of rotational diffusion that follows the DNA backbone, the ATP-bound MSH sliding clamps rotate freely while in discontinuous contact with the DNA (78). This makes their movement on DNA much like a washer on a screw. In addition, the diffusion coefficient of an ATP-bound sliding clamp increases at least 3-fold over a searching clamp, which with the lifetime dramatically increases the calculated coverage of an MSH on naked DNA by thermal motion alone to tens of thousands of nucleotides.

In addition to real-time SM imaging, genetic and biochemical observations suggest that multiple long-lived ATP-bound MSH hydrolysis-independent sliding clamps are the single most critical intermediates in initiating MMR. First, ATP binding- or hydrolysis-deficient MSH mutations located in the Walker A/B-binding motif retain strong mismatch binding activity, but are deficient for MMR (65, 82–86). Second, the ability to form a sliding clamp strictly correlates with biological function, whereas mismatch/lesion/structure binding is necessary but not sufficient for biological function (42, 43, 65, 83, 87–89). Finally, stoichiometry studies suggest that 4–8 MSH molecules appear associated with a single repair event *in vitro* (30, 31). The take-home lesson from these many observations is that when examining MSH function(s), one must develop biochemical conditions in which the formation of ATP-bound sliding clamps is robust and stable.

MLH/PMS real-time SM imaging studies have not yet clarified their actions. Using high-throughput DNA curtain technology, the ScMlh1-ScPms1 heterodimer was shown to occasionally form a clamp-like structure that was both capable of long-range hopping-sliding diffusion and adept at passing around a stable nucleosome (90). This observation contrasts the diffusion of MSH proteins that are blocked by nucleosomes (90), until a critical mass of stable ATP-bound sliding clamps are loaded, which are then capable of displacing the histone octamer (91, 92). When associated with ATP-bound sliding clamps, the diffusion characteristics of the ScMsh2-ScMsh6·ScMlh1-ScPms1 complex appeared similar to ScMsh2-ScMsh6 alone (93). A major puzzle is how these SM diffusion and interaction data mesh with atomic force microscopy observations showing compaction of the ScMlh1-ScPms1 heterodimer (62). An intriguing hypothesis would be that regulated compaction of MLH/PMS might alter the biophysical characteristics of the MSH sliding clamp promoting efficient and/or controlled downstream interactions along the DNA helix. This would effectively make MSH sliding clamps a stable but diffusible platform for MLH/PMS function(s).

A Framework MMR Model (Version 3.0)

The Molecular Switch MMR Model (Version 3.0) was proposed nearly two decades ago and solved the distant-initiation problem with simple 1D facilitated thermal diffusion (38, 47, 49, 95). The original concept was based on the hypothesis that ATP binding (not hydrolysis) drives conformational transitions in MMR components, which capture and ultimately utilize nor-

mally occurring thermal motions (termed: rectified Brownian motion (96)). The evidence that MSH proteins function as a mismatch-dependent molecular switch appears overwhelming (Fig. 2*A*). A transition of binding proteins to one-dimensional rotational diffusion along the DNA backbone is a well known mechanism that speeds a search process. However, mismatch-provoked ADP→ATP exchange by MSH proteins, which then results in the formation of a freely diffusible sliding clamp that is ~600-fold more stable on the DNA than a searching MSH, appears to fully satisfy the definition of a molecular switch undergoing allosteric-driven rectified Brownian motion (Fig. 2*A*).

For *E. coli*, a complete Molecular Switch Model predicts multiple ATP-bound MutS sliding clamps that provide stable platforms for MutL association (Fig. 2*B*, ***1***), and support controlled interaction with the downstream effectors such as MutH and UvrD (Fig. 2*B*, ***2***, ***3***, and ***4***). Following MutH activation (Fig. 2*B*, ***2***), the spontaneous turnover of the MutS·MutL·MutH incision-initiating complex was proposed (Fig. 2*B*, ***3***) because its function in MMR is complete. The loading of multiple ATP-bound MutS sliding clamps ensures that a second MutS·MutL complex is in place to form a complex with UvrD, which would be attracted to the incipient strand scission (Fig. 2*B*, ***4***). Like the MutSLH complex, ATP binding by MutL was proposed to stabilize the interaction between MutS·MutL and UvrD (Fig. 2*B*, ***4***). This would in effect make MutL a second molecular switch where ATP binding induces a conformational transition that controls downstream complex formation. One imagines that the interaction between UvrD and the MutS·MutL clamp complex on the DNA might enhance the processivity of its helicase unwinding activity much like PCNA enhances the processivity of replicative polymerases. UvrD unwinding would ultimately present an ssDNA end to an exonuclease (Fig. 2*B*, ***5***). This latter point is important because to date there have been no observed interactions between the *E. coli* exonucleases and the core MMR machinery. If/when spontaneous turnover of the MutS·MutL·UvrD complex results in its dissociation, a following sliding clamp complex may iteratively pick up where the last left off until the mismatch is excised and no additional MSH sliding clamps may be loaded (Fig. 2*B*, ***5*** and ***6***). It is the loading of multiple MSH·MLH/PMS complexes that ensures MMR is both dynamic and redundant such that repair is almost always faithfully completed.

The mechanism of MMR outside of γ-proteobacteria appears similar if not largely identical to that proposed above (Fig. 3). The first step is loading multiple ATP-bound MSH sliding clamps that then provide a platform for additional MMR component associations (Fig. 2*A*). For 5′-excision, a simple interaction between MSH2-MSH6 and EXOI (Fig. 3*A*, ***1***) (97–99) generates an excision tract (Fig. 3*A*, ***2*** and ***3***) that appears to be regulated by RPA (28–31). Bulk biochemical analysis of the human reaction has suggested that HsMLH1-HsPMS2, although not essential, plays a role in termination of the 5′-excision tract just past the mismatch (31). Such a function would be consistent with a controlling role for HsMLH1-HsPMS2.

In contrast, 3′-excision requires the MSH·MLH/PMS complex to interact with PCNA (Fig. 3*B*, ***1***) loaded at the 3′-strand scission to activate the MLH/PMS endonuclease (Fig. 3*B*, ***2***).

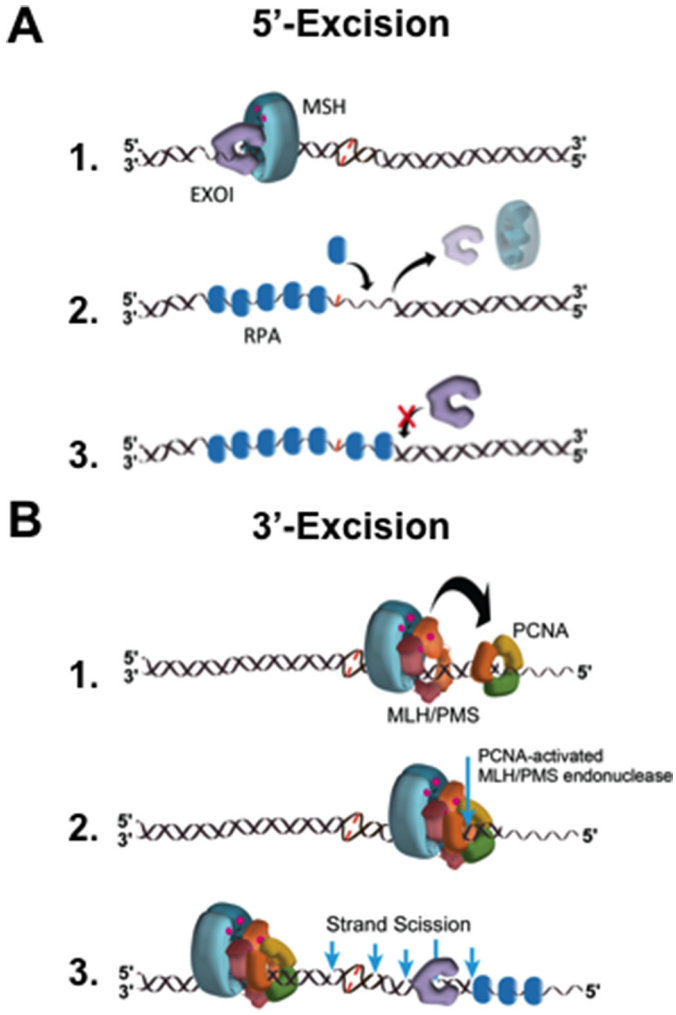

FIGURE 3. **The molecular switch model for eukaryotes.** The 5′- and 3′-excision reactions require different components, but both processes start with the loading of multiple ATP-bound MSH sliding clamps. *A*, 5′-excision. ***1***) An ATP-bound MSH sliding clamp interacts and stabilizes EXOI on the DNA at a 5′-strand scission and enhances its 5′→3′ exonuclease processivity. ***2***) When one MSH·EXOI complex spontaneously dissociates, a following MSH sliding clamp interacts with EXOI, restarting exonuclease digestion. ***3***) The binding of RPA to the nascent gap inhibits EXOI exonuclease activity until its association with a following MSH sliding clamp. This process is iterative until the mismatch is released, eliminating the loading of additional MSH sliding clamps (bottom gapped DNA). *B*, 3′-excision. ***1***) An MLH/PMS associates with an ATP-bound MSH sliding clamp that then diffuses together to PCNA bound to a 3′-strand scission (likely the 3′-end of leading strand replication). ***2***) The interaction between MSH·MLH/PMS and PCNA activates the intrinsic MLH/PMS endonuclease. ***3***) Diffusion of the MSH·MLH/PMS·PCNA complex (shown) or hand-off of the MLH/PMS to PCNA and diffusion of the MLH/PMS·PCNA complex (not shown) allows the MLH/PMS intrinsic endonuclease to introduce multiple strand scissions in the 5′-direction from the 3′-end that are substrates for the EXOI 5′-exonuclease. This process is iterative until the mismatch is released, eliminating the loading of additional MSH sliding clamps (bottom gapped DNA). See text for narrative.

Once activated, the MLH/PMS introduces multiple strand scissions between the 3′-end to just past the mismatch (Fig. 3*B*, ***3***). One can envision at least two types of dynamic structures: 1) the MLH/PMS might be sandwiched between the MSH and PCNA sliding clamps, which together provide some diffusion-controlled processivity to the endonuclease incisions, or 2) the MSH could hand off the MLH/PMS to PCNA, which alone could provide diffusion-controlled processivity to the endonuclease incisions. This latter possibility appears to be supported

by cellular imaging studies in *Saccharomyces cerevisiae*, where ScMsh2-ScMsh6 disappears after colocalization of ScMlh1-ScPms1 with ScPCNA (100). Regardless, once activated, the multiple MLH/PMS incision fragments each contain nascent 5′-ends that, at least *in* vitro, may be substrates for the EXOI 5′-exonuclease activity (Fig. 3*B*, ***3***). Like *E. coli*, iterative MMR complexes ensure that the reaction is dynamic and redundant until the mismatch is released and no additional MSH sliding clamps may be loaded.

Future Prospects

It is likely that real-time SM technologies will ultimately visualize the complete MMR process *in vitro* and *in vivo* to detail the mechanism(s) that animate repair. Perhaps the most intriguing unanswered problem still surrounds understanding the function(s) of MLH/PMS in MMR. In addition, visualizing the 3–4 components of the eukaryotic 5′-excision reaction would also seem ripe for real-time SM imaging. Finally, there still seem to be either missing factors or missing mechanisms in the eukaryotic MMR reaction. For example, an *exoI* mutation in *S. cerevisiae* is an extremely weak Mutator (30). However, the 5′→3′ specific EXOI exonuclease is required for *both* the eukaryotic 3′-excision and the eukaryotic 5′-excision reactions *in vitro*. This observation appears to underline a possible disconnect between the genetics and biochemistry of MMR that awaits resolution.

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